Attorney Docket No.: 5051-337DVCT3 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Stomp et al.
Application No.: 10/677,441
Filed: October 2, 2003
For: *Genetically Engineered Duckweed*

Confirmation No.: 9042
Group Art Unit: 1638
Examiner: Li Zheng

Date: November 28, 2006

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Submittal of Declaration of Dr. Lynne F. Dickey under 37 C.F.R. § 1.132

Attached is a Declaration under 37 C.F.R. § 1.132 as submitted to the USPTO on March 24, 2006 for U.S. Application Serial No. 10/273,974.

Attorney Docket No. 5051-337DVCT2 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Stomp and Rajbhandari
Application No.: 10/273,974
Filed: October 18, 2002
For: METHOD FOR PRODUCING STABLY TRANSFORMED DUCKWEED USING MICROPROJECTILE BOMBARDMENT (AMENDED)

Confirmation No.: 4514
Group Art Unit: 1638
Examiner: A. Mehta

Declaration of Dr. Lynn F. Dickey under 37 C.F.R. § 1.132

I, Dr. Lynn F. Dickey, hereby declare and say as follows:

1. I am the Vice President of Research at Biolex, Inc., the exclusive licensee of U.S. Application Serial No. 10/273,974 (*hereinafter*, "the '974 application"). Prior to joining Biolex, Inc., I was a Research Assistant Professor in the department of Botany at North Carolina State University, the assignee of the '974 application. In that capacity, my research program focused on plant gene expression with a concentration on mRNA stability and translation.

2. The study described below was conducted under my supervision and direction.

3. *Transformation of Lemna callus using particle bombardment.* Plasmid DNA (Egs05 in T7) was prepared using a Qiagen Maxi-Prep kit (Qiagen, Valencia, CA), and gold microcarriers (1.6μm) were coated according to the standard protocol (Biorad, Hercules, CA). The plasmid, Egs05 in T7, contains cassettes for expression of the neomycin phosphotransferase gene (NptII) and the β-glucuronidase gene (Gus) (Figure 1). Prior to particle bombardment, approximately 10 medium sized (5mm) pieces of *Lemna* callus were placed in the center of a petri plate containing callus maintenance medium (Murashige & Skoog medium + 3% sucrose, 0.15% Gelrite, 1 μM 2,4-D and 2 μM BA. The callus was bombarded with a Biorad PDS-1000/He following the standard protocol (Biorad, Hercules, CA) using three helium pressures: 900, 1100 and

2200psi. After bombardment, callus was incubated in the dark on callus maintenance medium for 48 hours. The callus bombarded at 900psi was then placed on callus maintenance medium containing 100µg/ml or 200µg/ml kanamycin (Kan100, Kan200). The callus bombarded at 1100 and 2200psi were transferred to 0.5x Schenk and Hildebrandt (SH) medium containing 1% sucrose and Kan100 or Kan200. Callus assayed for Gus expression by histochemical staining 72 hours after bombardment tested positive in all 3 treatments. Callus was grown under continuous light on solid 0.5x SH medium with 1% sucrose and began to generate frond tissue after 8 to 10 weeks. One transgenic frond line was harvested from each of the three helium pressure treatments and transferred to liquid 1x SH medium containing 1% sucrose for vegetative propagation. The 900 and 2,200psi lines were recovered from Kan100 selection while the 1,100 psi line was taken from Kan200 selection.

4. *Analysis of transgenic lines.* Frond tissue from the transgenic lines tested positive for Gus expression by a histochemical staining assay. Genomic DNA was extracted from the transgenic fronds as described in Dellaporta et al., (1983) *Plant Molec. Biol. Report.* 1:19-21. PCR was performed using standard techniques with 2 sets of oligos:

Biolex 24 + Biolex 112 – Amplifies fragment from Gus gene cassette (1852bp)
Biolex 510 + Biolex 511 – Amplifies fragment from NptII gene cassette (1107bp)

Oligos (5' to 3'):

Biolex 24: CGTAAGTTTCTGCTTCTACCTTTGATATAT
Biolex 112: GCTGCTCCACACATGTCCAT
Biolex 510: CAGCTGGTACATTGCCGTAG
Biolex 511: CACTGACTTCCATAAATTCC

For both sets of oligos, a positive control (Egs05 in T7 plasmid) and two negative control reactions (wildtype *Lemna* and "no DNA") were run in parallel with reactions for the transgenic lines. The expected PCR products were produced in

reactions with the 900, 1100, and 2200psi transgenic lines and in the positive controls (Figures 2 and 3, lanes 2 and 5-7). Wildtype *Lemna* and "no DNA" control reactions did not yield the amplicons (Figures 2 and 3, lanes 3 and 4).

A Southern blot was performed on XhoI digested DNA from wildtype *Lemna* and the 3 transgenic lines. The blot was probed with a digoxigenin (DIG) labeled Gus gene fragment and detected by chemiluminescence according to the manufacturers' recommendations (Roche, Indianapolis, IN). Multiple hybridization signals were detected in the 3 transgenic lines (Figure 4, lanes 3-5), while none were detected in wildtype *Lemna* (Figure 4, lane 2).

5. These results indicate that stable transformation of *Lemna* callus with integration into the genomic DNA was achieved using biolistic transformation essentially as described in the '974 application. The 900psi helium pressure used in the study described above is the closest setting available with the Biorad PDS-1000/He gene gun to the 800psi described in Example 20 of the '974 application. Importantly, the results demonstrate that biolistic transformation was achieved across a wide range of helium pressures.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Lynn F. Dickey, Ph.D.

24 Mar 06
Date